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Sensory characterisation of wines without added sulfites via specific and adapted sensory profile

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ABSTRACT

Aim: In the few past years, consumer expectation has shifted toward low-additive foodstuffs. In the wine industry, this has been evidenced by the development of wines without any added SO_2 during the winemaking process, including bottling. This has also led to the development of alternative methods to replace SO_2 for winemaking, which, alongside the dearth of studies on these new production methods, raises the question of the sensorial impact of sulfites and sulfite alternatives on wines after aging.

Methods and results: Wines were made from Merlot N. grapes at two different maturity levels, with or without SO_2 addition throughout the whole process. From the same batch, wines were also produced with bioprotection applied to the harvest only as an alternative to SO_2. Sensory evaluation was performed after two years of aging, with the development of specific and adapted training methods to determine the sensory profile of the wines. In this way, a high sensory proximity between wines without SO_2 (whether produced with bioprotection or not) was highlighted, and they were described as significantly different from wines with SO_2.

Conclusion: This approach demonstrated that, for expert tasters and despite the use of bioprotection, wines without SO_2 had specific sensory characteristics compared to wines with SO_2.

Significance of the study: This study was a first sensory step towards characterising wines produced without any added SO_2. In future work, it could be used to highlight chemical compounds associated with sensory descriptors discriminating between them.

KEYWORDS

wines without sulfites, bioprotection, sensory analysis, sensory profile, panel training

Supplementary data can be downloaded through: https://oeno-one.eu/article/view/3566

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INTRODUCTION

Sulphur dioxide (SO_2) is one of the most popular additives in the winemaking process, and can be added at different strategic stages: on the must, after fermentation (alcoholic and malolactic), during wine aging, and at bottling. Indeed, it possesses three main properties: antioxidant (Carrascón *et al.*, 2018; Waterhouse, 2012), antioxidasic (Dubernet and Ribéreau-Gayon, 1973; Ribéreau-Gayon *et al.*, 2017), and antimicrobial (Constanti *et al.*, 1998; Albertin *et al.*, 2014).

Nowadays, the reduction in the use of additives in agribusiness manufacturing processes is a societal demand, not specific to oenology. The use of sulfur dioxide (SO_2) can be harmful to humans, both for process operators when the usual precautions are not taken, and more generally for highly sensitive consumers. Indeed, it has been shown that exposure to sulfites can cause a range of adverse clinical effects in susceptible individuals (less than 10 % of the population), ranging from dermatitis to urticaria, including redness, hypotension, abdominal pain and diarrhea, as well as anaphylactic and asthmatic reactions (Timbo *et al.*, 2004; Vally *et al.*, 2009; García-Gavín *et al.*, 2012). Furthermore, in the context of global warming, an increase in wine pH implies lower SO_2 efficiency.

With the evolution of consumer expectations (Costanigro *et al.*, 2014; Amato *et al.*, 2017), new alternatives to SO_2 are emerging, particularly for harvest protection, such as bioprotection with the addition of yeasts. This concept of excluding chemical additions was inspired from the agri-food industry and has its own definition: the term "bioprotection" refers to the use of microorganisms or their metabolites to inhibit, or even eliminate, unwanted microorganisms in foods in order to guarantee the hygienic qualities of the products, and thus increase their life without altering their sensory properties (Stiles, 1996; Lücke, 2000).

In the literature, very few studies on the sensory impacts of SO_2-free winemaking processes or the use of alternatives are currently available. Morgan *et al.*, (2019) conducted numerous trials to evaluate the impact of the reduced use, or even suppression, of SO_2 on Pinot Gris microbiota and, to a lesser extent, its sensory characteristics, but without panel training. While Simonin *et al.* (2018) showed the efficiency of bioprotection using *Torulaspora delbrueckii* - which occupies the ecological niche - on Aligoté grapes, they did not develop an extensive sensory evaluation. In red wine, Benucci *et al.* (2018) highlighted the impact of two *Metschnikowia* species on wine volatile composition, but they did not evaluate the sensory characteristics; meanwhile, Simonin *et al.* (2020) could not conclude on the sensory impact of *Metschnikowia pulcherrima* in bioprotection, where again no panel training session was carried out.

Thus, a real sensory characterisation of wines produced without added sulfites and with the use of alternatives needed to be undertaken.

Descriptive sensory methods are commonly used for the sensory analysis of wines to characterise sensory differences between products after a first step of discriminative tests to highlight differences. These descriptive methods may use qualitative and/or quantitative approaches, and comprise a multitude of tests developed by food scientists, such as Flavor and Texture Profile (Szczesniak, 1963; Cairncross & Sjöström, 1997), Flash Profile and Free Choice Profiling (Williams & Langron, 1984; Sieffermann, 2000). All these tests have been adapted from the conventional descriptive analysis, also called the Conventional Sensory Profile, in line with NF ISO 11035: 1994. This test was based on the variation in individual perceptions with stimuli concentration, as described by Lawless (1999). At the same time, Lawless indicated that an independent intensity scale could not be adapted to the sensory analysis of a complex matrix and revealed the need to train the panel, which is too time-consuming for such a test. New approaches were studied to ensure that training phases were not required for these sensory tests (Lelièvre *et al.*, 2008). However, according to the norms of the conventional profile, a sensory test takes approximately 120 hours, including vocabulary generation, training and evaluation of training, before the sensory evaluation can be performed (NF EN ISO 13299: 2016). This was unworkable in practice, underlining the need to optimise rapid panel training for conventional descriptive analyses.

The objective of this study was to determine the sensory profiles of wines produced using different winemaking processes after 1.5 years of bottle maturing: a classical treatment with SO_2, another one with bioprotection and a last one without any treatment. In the global warming context, two levels of maturity were tested. A robust sensory analysis methodology, integral to this study, was implemented in order to reach these specific objectives. Thus, from the standard ISO sensory profile method, the goal was to develop a sensory profile specific to these wines, in which only

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the use of SO_2 and/or bioprotection varied. Moreover, as any preconceived result was expected, this method was adapted to be exploratory, with vocabulary generation and specific training.

MATERIALS AND METHODS

1. Wine production

1.1. General winemaking process

Merlot N grapes (*Vitis vinifera* L.) from the 2017 vintage were produced in the *Entre-Deux-Mers* area (Bordeaux, south-west France) on an estate using Organic Agriculture methods. They were harvested manually in small crates from the same plot, and the winemaking was carried out at the IFV (Institut Français de la Vigne et du Vin) facilities, in Blanquefort (France). The grapes were sampled to obtain homogeneous batches for the three winemaking processes, then crushed and destemmed. A pre-fermentation maceration at 10 °C was performed for 48 h before inoculation with 200 mg/L of commercial *Saccharomyces cerevisiae* (Actiflore ® F33) to perform the alcoholic fermentation and 10 mg/L of commercial *Oenococcus oeni* for the malolactic fermentation. The wines were aged for six months in stainless steel vats, then filtered and bottled in May 2018 (S2). Ethanol, total acidity, volatile acidity, pH, and free and total SO_2 via the Franz-Paul method (Paul, 1958) were measured at the moment of tasting (S1).

1.2. Winemaking protocols

Three alternatives were evaluated, using grapes at two stages of maturity (Maturity A at technological maturity and Maturity B harvested one week later) in a global warming context: 1, bioprotection in the form of 50 mg/L non-*Saccharomyces* strains of *Torulaspora delbrueckii* and *Metschnikowia pulcherrima* (Zymaflore ® Egide – Laffort)) was applied directly onto the grapes following the manufacturer's indications and without addition of SO_2 throughout the process; 2, similar to the usual practices, 50 mg/L of SO_2 was added at vatting, 30 mg/L of free SO_2 maintained during the wine ageing, and an extra 10 mg/L of SO_2 added at bottling; 3, no additions throughout the winemaking and ageing process.

2. Sensory approaches

2.1. General conditions

Sensory analyses were performed as described by Martin & De Revel (1999). All samples were analysed at controlled room-temperature (20 °C), in individual booths, using covered, black ISO glasses containing 50 mL of liquid (NF EN ISO 8589: 2010).

2.2. Sensory panels

Tasters were selected for the exercise on the basis of their availability and interest, and had equivalent homogeneous sensory expertise, because they had followed the same tasting training. Participants had not been informed about the characteristics of the study. They had all provided informed written consent.

➔ Panel 1 was made up of 24 tasters (18 women) with ages ranging from 22 to 50 years old (28.3 ± 2.7, mean ± SD). Participants were research laboratory staff from the Unité de Recherche Œnologie, Institut des Sciences de la Vigne et du Vin, Bordeaux University, with equivalent homogeneous, high sensory expertise.

➔ Panel 2 was made up of 11 tasters (8 women) with ages ranging from 22 to 24 years old (23 ± 0.4, mean ± SD). Participants were homogeneously trained in wine tasting (curricular sensory analysis training in Enology, 1st year Master's level) but none of them had previously taken part in the discriminative analysis panel.

2.3. Discriminative testing method

Discriminative testing was carried out to highlight product differences via triangle tests (NF ISO 4121: 2007). The triangle tests were performed by panel 1, by direct olfaction only.

2.4. Conventional sensory profile method

Descriptive testing was performed by panel 2 according to the conventional sensory profile (NF ISO 13299: 2016), divided into three successive steps. First, vocabulary generation was performed (NF ISO 11035: 1994) to get specific descriptors associated with the products' sensory space. In the second step, the panel was specifically trained in vocabulary generation (NF ISO 8586: 2014) and finally, the products were evaluated. All the tasting sessions were scheduled over one month.

2.4.1. Descriptor generation

Vocabulary generation was done in one session with wines in black ISO glasses coded with random three-digit numbers. All wines were simultaneously submitted to each taster with a specific and randomised order of tasting. Tasters were instructed as follows: "After the overall

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tasting (olfactory and mouth perception) of each wine, you will generate a list of ten descriptors. These descriptors must be precise, non-hedonic and definable. The ten chosen descriptors will be used to differentiate the wines, either by the intensity of character or by the presence or absence of character. These descriptors can be olfactory, gustatory or trigeminal.".

Next, all descriptors were pooled and discussed by the tasters in order to reach a consensus on which specific descriptors would be subsequently used. The discussion step was done without wines. Thirty minutes were dedicated to vocabulary generation and an hour and a half to the discussion.

2.4.2. Training testing methods

One hundred and ten descriptors were thus generated and fourteen were selected. References were defined for these descriptors in agreement with the consensus of the panelists, who then took training in them. Five specific one-hour training sessions were carried out.

Session 1: training for validation of references

The references used were often based on a simple everyday product, put into a hydroalcoholic solution at 12 %, v/v (Table 1). Diluted alcohol macerates were produced from absolute ethanol (analytical grade, 99.97 %/Merck, Darmstadt, Germany) and microfiltered water (Milli-Q Plus water system, resistivity 18.2 MΩ cm, Millipore, Saint-Quentin-en-Yvelines, France). Moreover, for all tasting training, participants had to use a nose clip to focus on taste and trigeminal perceptions for the mouth descriptors.

The purpose of the first session was to validate the selection of references for each descriptor. The tasters received each reference predefined during the descriptor generation task with a mention of the descriptor it was selected for. A discussion step was carried out afterwards to standardise the tasters' answers and validate the chosen references.

Session 2: training for validation and recognition of references

The first part of session 2 consisted in repeating session 1, while the second part was devoted to recognition. Tasters had to smell or taste samples, each of which they had to associate with a previously selected descriptor. Some descriptors could be repeated more than once. If the taster made a mistake, he or she had to repeat the sensory analysis on some of the descriptors.

Session 3: training in intensity evaluation

The tasters had to first repeat the recognition test as previously described. In addition, they had to carry out an intensity classification exercise for each descriptor. They received four samples, which they classified according to perceived intensity (NF ISO 8587:2007) and for which they determined the descriptor. If the taster made a mistake, he or she had to repeat this exercise on some of the descriptors.

For each descriptor, in order to vary intensities, different amounts of the corresponding macerate or pure chemical reference were added to Milli-Q H_2O (Table 1).

Session 4: Product characterisation training

In this session the tasters were trained under the conditions to be imposed in the final phase, in order to both evaluate the effectiveness of the training they had followed and to adjust the organisational "practicality" of the sensory analysis.

The panel received six wines. Among them, three were vinified with the same grapes as the wines evaluated during the generation of descriptors, one being evaluated twice. The other three wines were from the same variety (Chenet, J.-P., Merlot Vin Rouge de Pays d'Oc): one of the three wines remained as it was, 10 mg/L of acetaldehyde was added to the second to increase the "oxidation" descriptor, and 100 mg/L of tartaric acid was added to the third to increase the "acidity" descriptor. The panelists had to evaluate each descriptor as in the final wine evaluation.

Session 5: training for recognition of references in wine

In this last training session, the tasters received references in the same way as in the first session, but also in red wine (Chenet, J.-P., Merlot, Vin de Pays d'Oc). A sample of wine without any added reference was also provided.

2.4.3. Wine evaluation

The wines were evaluated using the sensory profile method in a single tasting session lasting one hour. This was performed in black ISO glasses coded with three-digit numbers. The wines were tasted according to a semi-monadic presentation. Olfactive descriptors were evaluated one by one

TABLE 1. Attributes and aroma reference standards employed for descriptive analysis training.

Descriptors	Definition	Composition for recognition session	Model solution for intensity session	Volume/Concentration in 12 mL of Milli-Q water for intensity training	
Red fruits	Strawberry, raspberry and currant (Odour)	Red fruits only of "Cocktails de fruits rouges" and "Fraises entières" (Picard)	Red fruits (300 g/L) and strawberry (100 g/L) in hydroalcoholic (12 % v/v)	1: 0.25 mL 3: 1 mL	2: 0.5 mL 4: 2 mL
Red fruit maturity	Fresh red fruits	Red fruits only of "Cocktails de fruits rouges" and "Fraises entières" (Picard)	Red fruits (300 g/L) and strawberry (100 g/L) in hydroalcoholic (12 % v/v)	1: 0.25 mL 3: 1 mL	2: 0.5 mL 4: 2 mL
	Jammy red fruits	Organic jammy red fruits (Leclerc)	140 $g.L^{-1}$ in hydroalcoholic (12 % v/v)	1: 0.25 mL 3: 1 mL	2: 0.5 mL 4: 2 mL
	Cooked red fruits	Red fruits only of "Cocktails de fruits rouges" and "Fraises entières" (Picard) cooked	Red fruits (300 g/L) and strawberry (100 Òg/L) in hydroalcoholic (12 % v/v)	1: 0.25 mL 3: 1 mL	2: 0.5 mL 4: 2 mL
Fresh blackcurrant	Blackcurrant (Odour)	Blackcurrant of "Cocktails de fruits rouges" (Picard) and "crème de cassis" (Cherry Rocher)	200 g/L in hydroalcoholic (12 % v/v)	1: 0.38 mL 3: 1.5 mL	2: 0.75 mL 4: 3 mL
Cooked black cherries	Cooked black cherries (Odour)	"Cerises noires dénoyautées" (Picard) cooked cherries	200 g/L in hydroalcoholic (12 % v/v)	1: 0.25 mL 3: 1 mL	2: 0.5 mL 4: 2 mL
Mint	Mint (Odour)	Organic essential oil, Spearmint (Florame)	2 drops in 1 L of H_2O Milli-Q	1: 0.13 mL 3: 0.5 mL	2: 0.25 mL 4: 1 mL
Black pepper	Black pepper (Odour)	Black Pepper (Ducros)	4 $g.L^{-1}$ in hydroalcoholic (12 % v/v)	1: 62.5 µL 3: 250 µL	2: 125 µL 4: 500 µL
Vegetables	Vegetables (without green pepper) (Odour)	*Elytrigia repens*, juice from canned asparagus (Daucy)	10 g/L in hydroalcoholic (12 % v/v)	1: 0.13 mL 3: 0.5 mL	2: 0.25 mL 4: 1 mL
Smoke	Smell of chimney smoke (Odour)	Chimney charcoal	15 g/L in hydroalcoholic (12 % v/v)	1: 0.5 mL 3: 2 mL	2: 1 mL 4: 4 mL
Oxidation	Oxidised apple and potatoes (Odour)	Acetaldehyde (Merck, Darmstadt, Germany), methional (Sigma-Aldrich, Australia)	In H_2O Milli-Q	1: 5 mg/L 3:15 mg/L	2: 10 mg/L 4: 20 mg/L
Acidity	Acidity (Taste)	Tartaric acid (Merck, Darmstadt, Germany)	In H_2O Milli-Q	1:10 mg/L 3:30 mg/L	2: 20 mg/L 4: 40 mg/L
Astringency	Astringency (Sensation)	Aluminum sulfate (Fisher Scientific, Hampton, USA)	In H_2O Milli-Q	1: 1 mg/L 3:10 mg/L	2: 5 mg/L 4: 15 mg/L
Bitterness	Bitterness (Taste)	Quinine sulfate (Merck, Darmstadt, Germany)	In H_2O Milli-Q	1: 0.5 g/L 3: 2 g/L	2: 1 g/L 4: 3 g/L
Coolness	Freshness (Sensation)	Organic essential oil, Spearmint (Florame)	2 drops in 1 L of H_2O Milli-Q	1: 62.5 µL 3: 250 µL	2: 125 µL 4: 500 µL

NB: the underlined references correspond to those used for the intensity session

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for all wines to ensure that the panelist were focused on only one descriptor, and compare for this specific descriptors all wines. For all mouth descriptors, the wines were evaluated one by one to limit sensory fatigue. One tasting booth was associated with one or two descriptors, except for the gustative descriptors which were distributed between two booths; thus a panelist had to evaluate the samples in eight booths. The wines had different codes with random three-digit numbers in each booth. For all booths, the order of presentation of the samples was randomised among the panelists and among the booths in a Latin square arrangement. Similarly, for all panelists, the order of the tasting booths was randomised in a Latin square arrangement. Evaluations in the mouth were carried out after the olfactive ones. Descriptor intensities were assessed on ten centimeters continuous bounded scales from "no intensity" to "high intensity" (NF ISO 4121: 2003).

3. Data analysis

3.1. Statistical treatments

All statistical treatments, except hierarchical clustering, were performed using the Rstudio software (Rstudio Inc., Boston, USA, 2018). The XLSTAT software (Addinsoft, Paris, France, 2018) was used for hierarchical clustering.

3.2. Vocabulary selection

For each descriptor generated by the panelists, citation frequency was calculated by dividing the number of times it was used by the total number of answers by all the panelists. The thirteen most used terms were selected as descriptors for these wines.

3.3. Analysis of panel performance

Standardised principal component analysis (PCA) was performed to analyse panel performance, in particular to study the consensus between panelists. It was used with tasters representing variables and wines representing individuals. Different specific parameters of PCA were analysed, including the eigenvalue of dimensions to evaluate the impact of inter-individual variability and consensus, as well as the contribution and cos^2 of variables to explain the dimensions to evaluate, the representation of which the tasters influenced.

3.4. Product characterisation

Univariate analysis was used to analyse the results of sensory profiles. A two-way Friedman non-parametric statistical test was used to analyse the interaction between modality and maturity parameters with a post-hoc Nemenyi test associated with the Friedman test. The impacts of modality and maturity were also studied independently via a Wilcoxon non-parametric test (two and multi sample comparison).

Hierarchical clustering analysis was used to evaluate the similarity between products; and the clusters were analysed to specifically search which descriptors influenced cluster formation.

RESULTS AND DISCUSSION

1. Discriminative test

For each maturity level, all the wines were compared two by two by panel 1 via an olfactory triangle test. The results (Table 2) showed significant differences (pvalue < 0.05) between all modalities harvested at technological maturity. However, for advanced maturity, "Without SO_2" and "Bioprotection" treatments were not differentiated.

TABLE 2. Olfactory impact of different winemaking processes by maturity.

		SO_2	Without SO_2	Bioprotection
Technological maturity	SO_2		***	**
	Without SO_2	***		*
	Bioprotection	***	*	
Advanced maturity	SO_2		**	*
	Without SO_2	**		=
	Bioprotection	*	=	

NB: ***, 0.1 % significance level; **, 1 % significance; *, 5 % significance level; = no significant difference according to binomial distribution.

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2. Wine description

2.1. Selected descriptors

The six wines were presented together to each of the eleven tasters in panel 2, who had to give ten descriptors which best illustrated all the sensations perceived. In this way, a total of one hundred and ten descriptors were generated. A first selection step was performed to eliminate hedonic terms, descriptors related to intensity and unsuitable descriptors as described by the vocabulary generation standard (NF ISO 11035: 1994). One hundred and seven descriptors were selected and three eliminated. These one hundred and seven descriptors were enumerated in order to determine their citation frequency and thirty-seven different descriptors were thus observed. The most cited terms were selected to continue the work. We considered the most cited descriptors to be those generated by at least one third of the panelists, equivalent to a citation frequency of 0.037 or higher (Figure 1). In this way, the thirteen most frequently used descriptors to characterise the set of wines were elicited. Among these descriptors, nine were direct olfactory descriptors; two were mouth descriptors ("Bitterness" and "Acidity"); and two were sensation descriptors ("Astringency" and "Tannic Quality"). The diversity of terms showed that the wines had a wide range of characteristics, from diverse fruity characters, like "Cooked black cherries" and "Fresh blackcurrants", to spicy characters, such as "Black pepper" or "Smoky". This approach elicited the most strongly perceived descriptors, whether they were the most intense (Campo *et al.*, 2010) or the most recognisable for the tasters.

A discussion was then held with the tasters to determine the consensual descriptors which would be used thereafter. The "Astringency" character was divided by the panelists into two descriptors: "Astringency" and "Tannic quality". Considering that many studies have highlighted that the mint descriptor is used to describe the minty odour, as well as coolness (Westerink and Kozlov, 2004), both "Mint" and "Coolness" were finally selected to evaluate this perception. The panel decided to

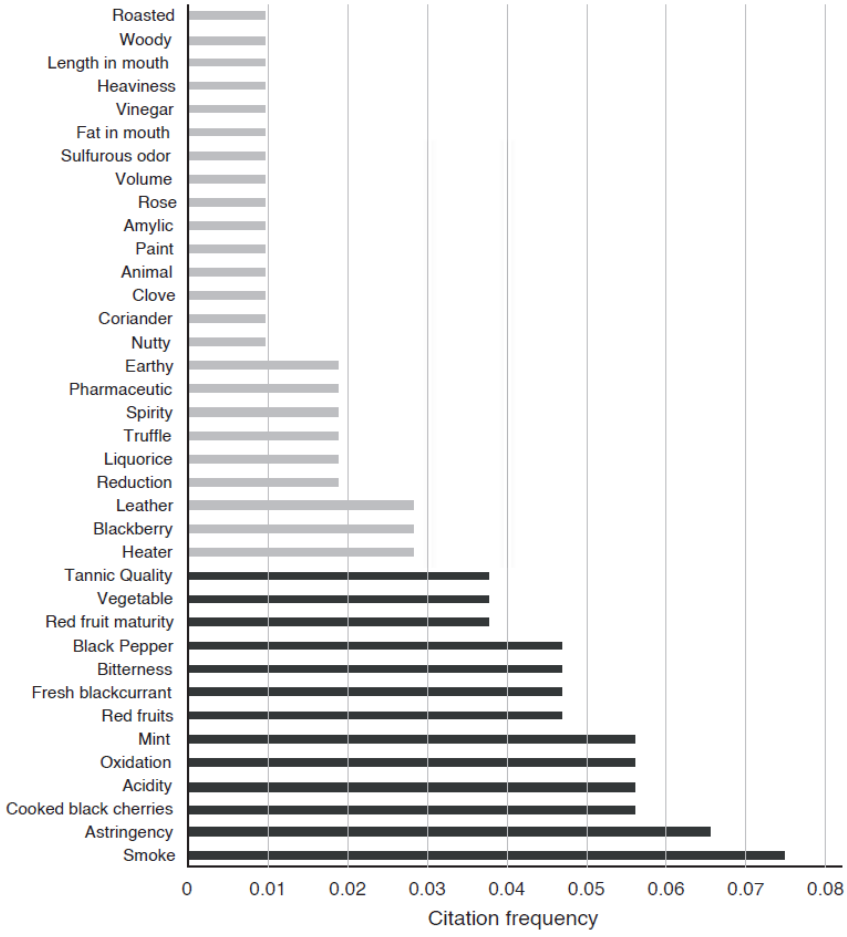


FIGURE 1. Wine descriptor citation frequency.

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use the descriptor "Red fruit maturity" to specify the quality of the red fruit notes perceived in wines. This descriptor was therefore not associated with an intensity scale, and the panelists decided to create a scale ranging from freshness to cooked character of fruit. The other selected descriptors were kept without any modification. Finally, fourteen descriptors (with "Coolness" added) were validated for further training and for the final sensory profile.

2.2. Reference choice

References for the thirteen selected descriptors were chosen following a discussion with the panel. Each panelist had his or her own olfactory vision of each descriptor in light of his or her previous life experience (Giacalone *et al.*, 2015); a stimulus can therefore give rise to different quantitative and qualitative responses from one subject to another (Lawless, 1999; McEwan *et al.*, 2002), resulting in non-consensus. Indeed, it is important to select the most appropriate sensory descriptors (Murray *et al.*, 2001) and to generate descriptors by qualified panelists, who in this case are students initiated in tasting (Guerrero *et al.*, 2001). Three references used for the trigeminal and taste descriptors, "Astringency", "Bitterness" and "Acidity", were selected in line with Chira *et al.* (2011): aluminum sulfate, quinine sulfate and tartaric acid respectively. For some descriptors, especially those related to fruits, the tasters agreed on the choice of references. Conversely, for the "Smoke" descriptor, the panelists struggled to define it and to come to an agreement. Therefore, in session 1, they selected references closest to it from a range of different references, which they received raw and in 12 % (v/v) dilute alcohol macerate. It was essential for the raw and macerate references to be similar in order to train the panel in intensities thereafter. During all the sessions, work on references and their adaptation was performed to produce the best possible references for the panel training.

During the first session, the panel validated references for the descriptors "Fresh blackcurrant", "Cooked black cherries", "Black pepper", "Vegetable" (with two references that were cited during the generation of descriptors: cut grass and asparagus) and "Oxidation".

Trigeminal and taste references were present in diluted alcohol solution 12 % (v/v), as described by (Chira *et al.*, 2011). After the first session, the panelists noted an interference due to ethanol. In the second session, the same references were presented in aqueous solution and were thus validated by the panelists. This illustrates the modification of perception of ethanol as a function of its concentration and the taster's sensibility (Mattes & DiMeglio, 2001).

For the "Red fruit maturity" descriptor, in the first session three references were presented to mimic a gradient from red fruits to cooked red fruits. This gradient was validated by the panel. However, they found that raspberry was too present and strawberry was absent. Accordingly, the panel validated the new mixture by incorporating strawberry during session 2.

The "Mint" descriptor was not properly represented by the mint macerate, which was too similar to mint tea. During session 2, a reference based on essential oil (Picard *et al.*, 2016) was presented to the panel, both for "Mint" (direct olfaction) and "Coolness" (sensation), and was validated.

For the "Smoke" descriptor, tasters had to choose among four references: two macerates of wood chips, a macerate with chimney ash and a macerate with charcoal. Unanimously, the panel chose the charcoal reference.

Finally, for tannic quality, tissue samples were presented to the panelists with tissues ranging from a high fineness to a rough texture. This corresponds to different qualities of tannins ranging from silky to coarse (Vidal *et al.*, 2004).

In the last training session, the references in the wines were changed; methional was chosen for the "Oxidation" descriptor in view of the sensory profile training carried out in session 4, because acetaldehyde was perceived by the panel as a vegetable in the wine matrix. Moreover, for the "Fresh Blackcurrant" descriptor, blackcurrant macerate could not be perceived in the chosen wine matrix. Initially, a blackcurrant syrup was used, in line with Campo *et al.* (2010) and Rigou *et al.* (2014), but its addition gave the wine an odour of caramel, rather than of blackcurrant. Finally, the addition of diluted blackcurrant cream turned out to be a better reference.

2.3. Training

The purpose of the fourth session was to increase the panel's awareness of the implementation of the final test and to get some initial results on the effectiveness of training, which we were able to partially validate. As a reminder, one red wine was repeated twice (to obtain repeatability), while two other red wines were modified (one acidified

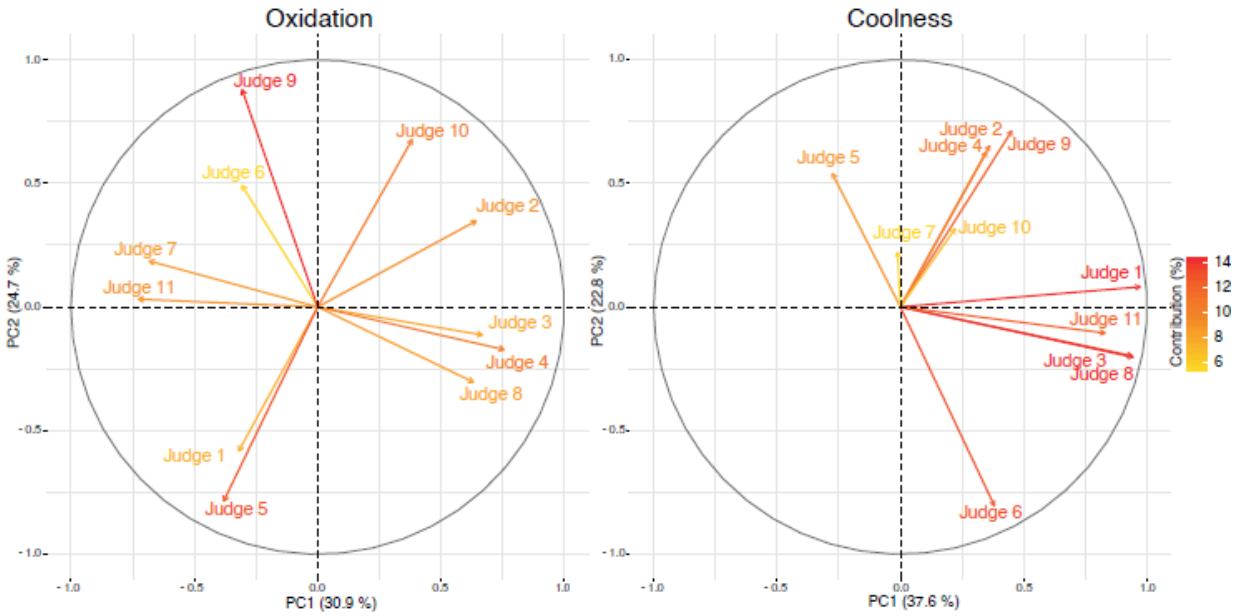


FIGURE 2. Example of correlation circles from PCA with non-consensus ("Oxidation") and with consensus ("Coolness") among panelists on the final sensory profile.

with tartaric acid and the other with the addition of acetaldehyde to increase oxidation). The result (not shown) indicated good repeatability for the rating of descriptors by the panelists, except for certain descriptors like "Red fruit maturity" and "Red fruits". This could be explained by a non-consensus on these descriptors. The wine with the addition of acetaldehyde was perceived as more "Vegetable" by a panel consensus. However, in the literature, this molecule is described by a rancid apple aroma, characteristic of oxidised wines (Zea *et al.*, 2015; Coetzee *et al.*, 2016). Following these results, references to the "Oxidation" descriptor were replaced by methional, which is characteristic of oxidised wines (Bueno *et al.*, 2010). The tasters did not find that the other wine (added tartaric acid) was more acidic. This could be explained by a long wait and a loss of concentration of the tasters, resulting in a non-consensus and no perceived difference between wines; the tartaric acid concentration may also not have been high enough for them to perceive the difference. Indeed, only two posts were devoted to taste descriptors, which created high expectations. In view of the process, this allowed us to readjust the organisation for the final sensory profile by doubling the positions for taste descriptors.

2.4. Interindividual descriptor consensus

These results were derived from the final session, that is, the sensory profile of the six wines in this study. As a function of the thirteen descriptors evaluated to create the sensory profiles of the six wines, different patterns of interindividual consensus were observed. As an example, Figure 2 shows the loading of the panelists on the first two principal components of the PCA performed on two selected descriptor intensities. An almost total interindividual consensus was observed for the "Coolness" descriptor (Figure 2). All panelists, except one, were on the positive side of the first axis, which represents 37.6 % of total variance, indicating that they tended to score wines similarly. The second axis represents 22.8 % of total variance, indicating that interindividual differences exist in descriptor evaluation, despite the training. In addition, seven panelists out of eleven showed high contribution (> 10 %) for this representation in two dimensions, especially panelists 1, 3, 8 and 11, who made a high contribution correlating to the first axis, thus representing interindividual consensus. It is possible to conclude from this result that the "Coolness" descriptor was consensual. For the "Oxidation" descriptor (Figure 2), five panelists were on the positive side of the first axis and six were on the negative side. Moreover, all panelists made a high contribution, indicating that they tended to score wines in different ways for this descriptor. Thus, it is possible to conclude that there was a non-consensus for this descriptor.

Overall, a non-consensus between panelists was observed for six out of the fourteen descriptors: "Black pepper", "Vegetable", "Oxidation", "Acidity","Bitterness" and "Tannic Quality". This non-consensus could be explained by unsuitable

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training for these descriptors, with the use of unsuitable references, or by the fact that the wines did not sufficiently express these descriptors for the panel to detect them. Nevertheless, all non-consensual descriptors were tested to research differences between the products and not one was found. More particularly, for the "Oxidation" descriptor, during the generation work, the panelists described oxidation character as "Oxidised Apple" which is one criteria of red wine oxidation, but it does not correspond to all the odours of red wine oxidation. In fact, it is possible for the polymorphism of red wine oxidation to impact tasting and to highlight an inaccurate descriptor.

For the eight other descriptors, a large majority of tasters were on the positive side of the first axis with high contribution, it was therefore possible to conclude that there was interindividual consensus for these eight descriptors (Figure 3). However, the fact that one or more tasters were on the opposite side of the first axis showed that they gave an opposite descriptor intensity score to that given by the majority of tasters. The descriptors were then evaluated after the elimination of that single taster (who had a maximum negative impact on the representation).

3. Product characterisation

Finally, a product characterisation was carried out in accordance with the eight consensual descriptors. Interactions between harvest protection treatment and maturity level were evaluated using an ANOVA test. No interactions were observed, and thus these two parameters were analysed separately.

3.1. Univariate analysis

3.1.1. Impact of grape maturity

Four descriptors were significantly impacted by the grape maturity level. As shown in Figure 4, wines from grapes harvested at technological maturity were described as being significantly more intense in terms of "Red fruit" notes, as well as "Coolness". Wines from grapes harvested seven days later were significantly more "Astringent" and showed a riper red fruit character. These observations are generally in agreement with those of Trujillo *et al.* (2019), who also observed with Merlot wines that at advanced maturity, fruity aromatic expression was modified, with a decrease in fruity intensity and fresh fruit character. Our results bring additional data regarding the same impact on the perception of coolness. Finally, assessing the reasons why astringency increases with maturity level is quite difficult, because the perceived astringency is the result of the evolution of many parameters, such as polyphenol extractability, concentration in seeds and skins, and changes to the organoleptic properties.

3.1.2. Impact of pre-fermentation treatment

Five descriptors were significantly affected by pre-fermentation treatment: "Fresh blackcurrant", "Cooked black cherries", "Smoke", "Mint" and "Coolness" (Figure 5). Four of these descriptors were more intense in wines without sulfites and/or wines with bioprotection. The "Smoky" character was the only one that was more intense for sulfited wines. This descriptor has also been described by Morgan *et al.* (2019) as specific to the use of sulfites in Pinot Gris. Moreover, for all descriptors, the wines from the winemaking processes which did not receive any sulfites, whether "Without sulfites" or "Bioprotection", were not discriminated by sensory analysis. The "Mint" and "Coolness" descriptors characterised wines without SO_2 winemaking itineraries. However, the bioprotection wines expressed a more intense "Fresh blackcurrant" character than the sulfited wines. While no key compound has yet been identified in red wines to explain the blackcurrant character, various compounds linked to or enhancing this aroma have been described. Some esters, such as ethyl propanoate, ethyl 2-methylpropanoate, ethyl 2-methylbutanoate (Pineau *et al.*, 2009) or 2-hydroxy-4-methylpentanoate (Lytra *et al.*, 2012), are involved in the blackberry aroma. Meanwhile, some volatile thiols, such as 4-mercapto-4-methyl-2-pentanone (4MMP), 3-(mercapto)hexyl acetate-3MHA) and 3-mercapto-1-hexanol (3MH), have been linked to the blackcurrant character by Bouchilloux *et al.* (1998) and Rigou *et al.*, (2014). Non-*Saccharomyces* yeasts can increase fruity aromas by the release of aromatic molecules through enzymatic activities, including β-lyase (Tominaga *et al.*, 1998; Esteve-Zarzoso *et al.*, 1998; Gonzalez *et al.*, 2012), which is involved in the production pathway of volatile thiols. *Metschnikowia pulcherrima* is more able to release 4MMP and 3MH than *Torulaspora delbrueckii* (Zott *et al.*, 2011; Sadoudi *et al.*, 2012). Other studies have shown that dimethyl sulfide has an impact on blackcurrant aromas through perceptual interactions in low concentrations (Escudero *et al.*, 2007; Lytra *et al.*, 2014). However, the dimethyl sulfide formed during alcoholic

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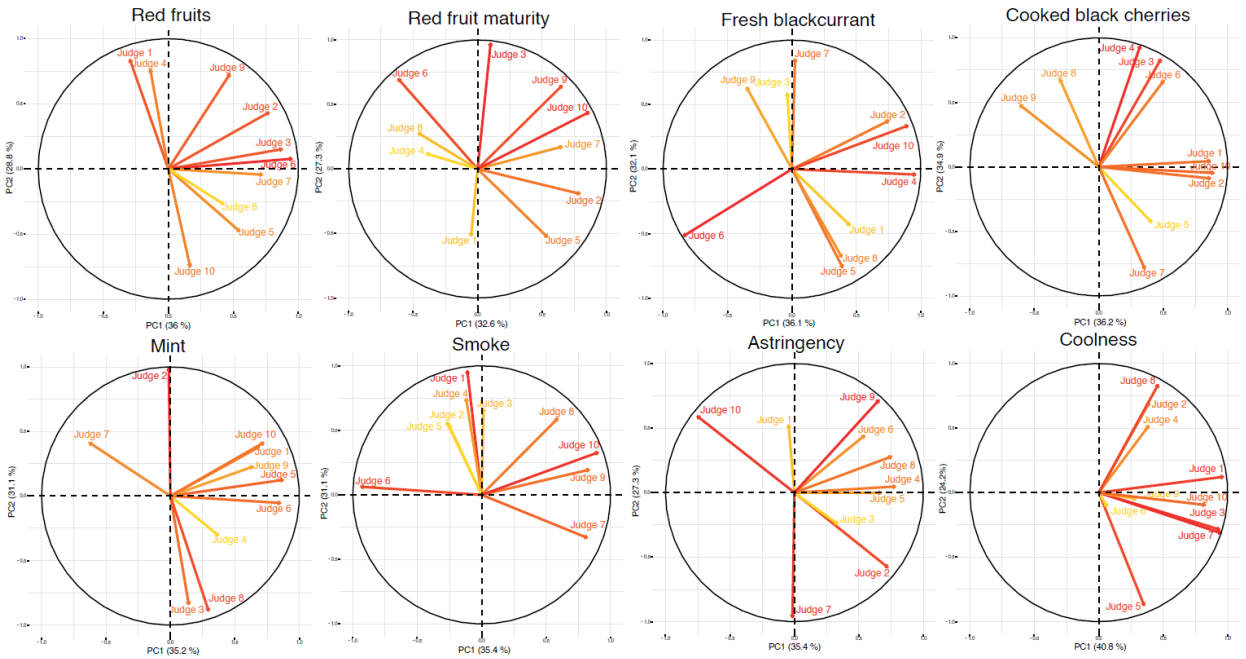


FIGURE 3. Correlation circles from the PCA with consensus of panelists on the final sensory profile without one taster.

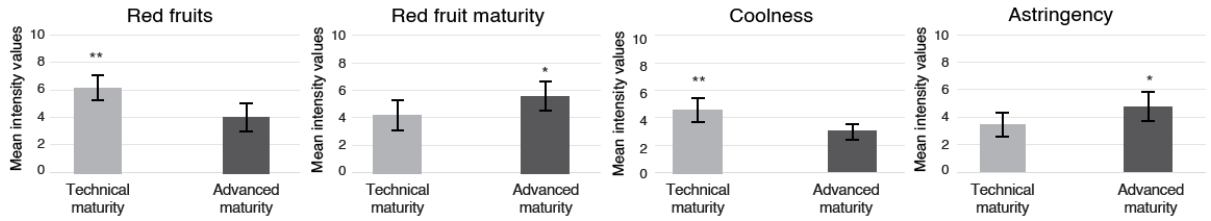


FIGURE 4. Descriptor intensities in wines from different grape maturity levels (modalities combined, n = 3). Error bar represents the confidence interval with a threshold of 0.05.

NB: **, 1 % significance; *, 5 % significance level according to Wilcox comparison test

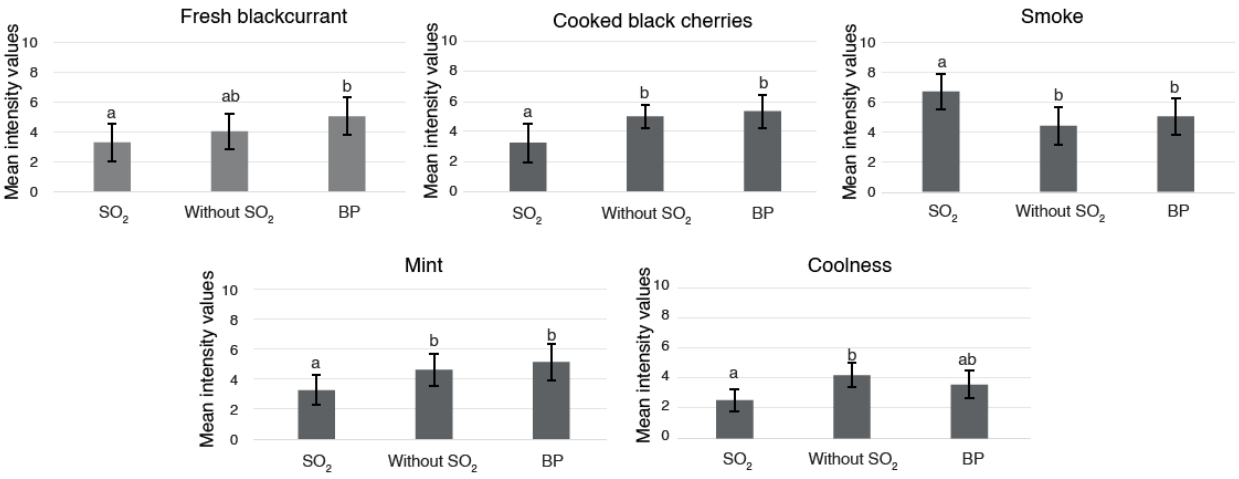


FIGURE 5. Descriptor intensities in wines from different pre-fermentation treatments (maturity levels combined, n = 2). Error bar represents the confidence interval with a threshold of 0.05.

NB: Values marked with a different letter are significantly different (Friedman test and Nemenyi comparison test for $\alpha < 5$ %)

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fermentation (therefore by yeasts (Deed *et al.*, 2019)) is mainly eliminated because this molecule is very volatile. Its concentration after 1.5 years is around 10µg/L (Segurel *et al.*, 2004) and has an impact on blackcurrant aromas. In the literature, studies have already shown the sensory impact of non-*Saccharomyces* yeasts in bioprotection: in particular, the use of *Lachancea thermotolerans* with *Lactobacillus plantarum* has produced wines of Tempranillo with more pronounced raspberry and blackberry fruit notes (Rubio-Bretón *et al.*, 2018), whereas the use of *Metschnikowia pulcherrima* brought more roudness and amylic aroma (Simonin *et al.*, 2020).

3.2. Multivariate analysis

Clustering was performed to characterise the proximities between wines. Figure 6 shows that the first grouping differentiates wines into two clusters, corresponding on the one hand to wines with sulfites and on the other to wines without sulfites. This second grouping did not differentiate bioprotection from non-sulfited wines, but did differentiate wines according to their harvest maturity level. Regarding SO_2 intake, wines from bioprotected grapes had not been sulfited throughout the winemaking, aging and bottling processes, which make them closer to the without-sulfite wines than to the sulfited ones. Overall, our sensory approach allowed us to gather wines, firstly, according to the presence of added sulfur dioxide, and secondly, according to the corresponding harvest maturity level. Thus, our results show that sulfite addition has a higher impact on the wines' sensory characteristics during the winemaking process than on the harvest maturity level, with the use of grape bioprotection being less discriminative.

CONCLUSION

The objective of this study was to set up an adapted sensory analysis methodology in order to describe fine sensory differences between wines produced using different winemaking processes and with different grape maturity levels after bottle maturing.

This work highlights the importance of choosing references and performing adequate training on the various descriptors to be able to get relevant results for the sensory profile. The training was essential to "normalise" the panel and obtain a better rating consensus for final descriptive evaluation. To complete the training, additional sessions could have been carried out to better accommodate the different descriptors on a wine matrix for the panel.

Specific features have been highlighted regarding the use of SO_2, bioprotection by adding non-*Saccharomyces* to the harvest, and a winemaking process without SO_2. Wines from grapes harvested at technological maturity

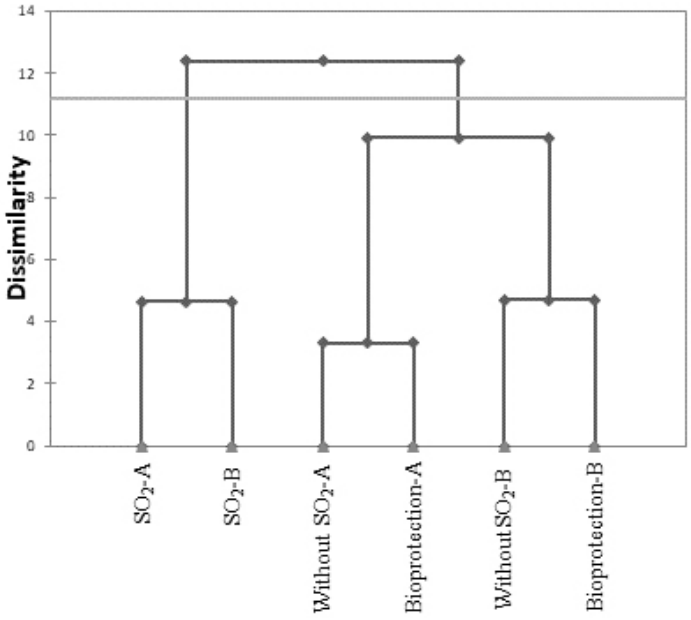


FIGURE 6. Clustering of wines with selected descriptors.
A corresponds to technological maturity and B to advanced maturity

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had more intense red fruit notes, while wines from grapes with advanced maturity were found to have greater jammy fruit notes and astringency. Wines without SO_2 were distinct from sulfited ones and were characterised by freshness (mint and coolness) and notes of cooked black cherries; bioprotected wines by fresh blackcurrant; and those with SO_2 by smoke. Hierarchical clustering applied to sensory data led to a significant differentiation between wines produced with and without any treatment.

This study is a first step in the characterisation of wines without added sulfites after bottle maturing. Indeed, it paves the way to the identification of underlying chemical markers. Lastly, a low but significant impact of bioprotection on the sensory specificities of wines without sulfites after aging was highlighted.

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Authors' Contributions

The authors Edouard Pelonnier-Magimel and Sara Windholtz contributed equally.

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